A. FRONT COVER/TITLE PAGE

TITLE OF RESEARCH PROJECT:

ASSESSING THE EFFECTS OF SOIL HUMIC AND FULVIC ACIDS
ON GERMINATION AND EARLY GROWTH
OF NATIVE AND INTRODUCED GRASS VARIETIES

NAME OF PRINCIPAL INVESTIGATOR: SENESI NICOLA-PROFESSOR

NAME OF CONTRACTOR: UNIVERSITA' DI BARI

CONTRACT NUMBER: N62558-05-P-0179

PURCHASE REQUEST NUMBER: W90C2K9889EN01

1st INTERIM REPORT

REPORT PERIOD: MARCH 22, 2005-SEPTEMBER 22, 2005

END OF FRONT COVER/TITLE PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	6 December 2005	1ST Interim Report, March 22, 2005 – September 22, 2005

4. TITLE AND SUBTITLE

ASSESSING THE EFFECTS OF SOIL HUMIC AND FULIC ACIDS ON GERMINATION AND EARLY GROWTH OF NATIVE AND INTRODUCED GRASS VARITIES, 1ST INTERIM REPORT

5. FUNDING NUMBERS

Contract Number N62558-05-P-0179

6. AUTHOR(S)

Principal investigator: Senesi Nicola-Professor

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Dipartimento di Biologia e Chimica Agroforestale ed Ambientale (DiBCA), Università di Bari., Via Amendola 165/A, 70124-BARI, ITALY

8. Performing Organization Report Number

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES

Engineer Research and Development Center , Cold Regions Research & Engineering Laboratory, 72 Lyme Road, Hanover, New Hampshire, 03755-1290, USA.

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

A

ABSTRACT (Maximum 200 words)

SCIENTIFIC WORK DONE DURING THE REPORTING PERIOD

Chemical and Spectroscopic Characterization of Original Soil Humic Acids

Experimental

The six humic acids (HAs) isolated by the USDA St.Paul group in duplicate from the two Wyoming soils, **Guernsey North (GN1 and GN2) and Guernsey South (GS1 and GS2)**, and one Utah soil, **Dugway (D1 and D2)**, object of this research, were characterized for their moisture and ash contents, elemental (C, H, N, S, O) and acidic functional group composition, and by Fourier transform infrared (FT IR) spectroscopy and fluorescence spectroscopy in the emission, excitation and synchronous scan modes.

RESEARCH PLANS FOR REMAINDER OF THE CONTRACT PERIOD

For the remainder of the contract period (20 months) research plans are the following:

(a) Experiments on the germination and early growth of the two introduced varieties Vavilov and SERP-select Siberian wheatgrass as affected by the three HAs, D-HA, GS-HA, and GN-HA.
(b) Chemical and spectroscopic characterization of HAs isolated from greenhouse soils.
(c) Experiments on the germination and early growth of the four grass varieties, alone or in combination (based on the growth differences found in the initial studies conducted at CRREL), as affected by the greenhouse soil HAs.
(d) Follow-up experiments with HA concentrations optimal to promote the growth of the four grass varieties.
(e) Correlation of the germination and seedling growth data with chemical and physico-chemical parameters of the HAs examined, in order to find out the HA parameters influencing germination and growth of the plant varieties examined.

14. SUBJECT TERMS

Navy, Italy, Soil Humic acids, Soils, Greenhouse, Acidic functional group, Wheatgrass, Germination

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19, SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

B. BODY OF THE REPORT

(1) SCIENTIFIC WORK DONE DURING THE REPORTING PERIOD

1. Chemical and Spectroscopic Characterization of Original Soil Humic Acids

1.1. Experimental

The six humic acids (HAs) isolated by the USDA St.Paul group in duplicate from the two Wyoming soils, **Guernsey North (GN1 and GN2) and Guernsey South (GS1 and GS2)**, and one Utah soil, **Dugway (D1 and D2)**, object of this research, were characterized for their moisture and ash contents, elemental (C, H, N, S, O) and acidic functional group composition, and by Fourier transform infrared (FT IR) spectroscopy and fluorescence spectroscopy in the emission, excitation and synchronous scan modes.

1.2.Results and Discussion

The elemental composition (C, H, N, S, O), calculated atomic ratios and acidic functional group contents, on a moisture- and ash-free basis, of the n. 6 original soil HAs described above are shown in Tables 1, 2, and 3. The contents of each element, atomic ratios and contents of acidic functional groups are within values typical of soil HAs. Although some significant difference is apparent for moisture and ash contents, no significant differences are shown for each element and acidic functional group content between the duplicates of each soil HA.
The FT IR spectra of the n.6 soil HAs are shown in Fig. 1. In general, they are similar to FT IR spectra of typical soil HAs. The spectra of the duplicates GN-HAs and GS-HAs only differ in the relative intensity of the band at around 1110 cm^{-1}, possibly attributed to polysaccharide-like tructures. No other relevant difference is apparent between spectra of duplicate HA samples.
The fluorescence emission, excitation and synchronous scan spectra of the n. 6 soil HAs examined are shown in Figs. 2, 3, and 4, respectively. Also fluorescence spectra are similar to those of typical soil HAs. They do not present any relevant difference between duplicate HAs, and are not very informative.

2. Germination and Early Growth of the Pryor and SERP-Select varieties of Slender Wheatgrass

2.1. Experimental

Each duplicated HA samples were mixed to obtain n. 3 final HA samples, i.e., **GN-HA**, **GS-HA** and **D-HA**, to be used at concentrations of 10 and 100 mg/L in the germination and early growth experiments of two varieties, cv. Pryor and germplasm line SERDP-select, of the native plant species slender wheatgrass.
Seeds were preliminary surface-sterilized by dipping them for 15 min in sodium hypoclorite 0.2 %, and then washing several times with distilled water. Twenty (20) seeds for each of the five (5) replicates were placed in Petri dishes on filter paper, and added with suspensions of each HA at each concentration in distilled water, or with distilled water only (control). The Petri dishes were kept in the dark for 6 days in a thermostated chamber at a temperature of 20 °C. After this time period, germinated

seeds were removed and counted, and the lengths of the primary root and shoot were measured.
After the end of the germination experiment and collection of germination data, the germinated seeds (seedlings) of the two slender wheatgrass varieties were inserted into holes of aluminum lids placed on the top of glass pots (5 seedlings per pot). The pots were filled with the Nitch nutrient solution, in the absence (control) or presence of each HA at concentrations of 10 and 100 mg/L. The pH of the nutrient solution was preliminarly adjusted to 6.5 with a solution of NH_4OH. Blanks (without seedlings) were also prepared for each treatment in order to measure the pH change during the growth period in the absence of plants. The pH of all treatment media ranged between 6.5 (control) and 5.9 (GS-HA at 100 mg/L). The pots were then placed in a Phytotron growth chamber, and seedlings were allowed to grow for a period of 21 days in the following conditions: (a) photoperiod of 12-h; (b) temperature of 20 °C and humidity of 74% during the illumination period; and (c) temperature of 17 °C and humidity of 70% during the dark period. At the end of the experiment, the pH of the growth solutions and blanks, and the length and fresh and dry weights (60 °C for 48 h) of roots and shoots were measured. All experiments were conducted in five replicates.
All germination and growth data were analyzed statistically by one-way analysis of variance (ANOVA) and the means of the treatments were separated by the least significant difference (LSD) test.

2.2. Results and Discussion

2.2.1. Germination data

Statistical treatment of data by one-way analysis of variance shows, with respect to the corresponding controls, and as a function of either the HA type or HA concentration (Table 4), the existence of: (a) no significant difference in the germination percentages (%) of the two slender wheatgrass varieties; (b) a highly significant difference in the length of primary shoot of germinated seeds of the Pryor variety; and (c) a highly significant difference in the lengths of both primary shoot and root of germinated seeds of the SERDP-select variety. However, numerical data in Table 5 and Fig. 5 suggest that the three HAs at both concentrations exert a general slight positive effect in promoting germination of both slender wheatgrass varieties.
Numerical data in Table 6 and Fig. 6 suggest that, with respect to the control, the primary root length: (a) is not affected by D-HA and GS-HA but is slightly increased by GN-HA at both concentrations, for the Pryor variety; and (b) is not affected by GS-HA but is increased by D-HA and GN-HA at both concentrations, for the SERDP-select variety. Further, with respect to the control, the shoot length of both varieties is enhanced at various extent by any HA at both concentrations.
When comparing data in Table 6 and Fig. 6 between the two varieties, it appears that the D-HA exerts a slightly greater positive effect on the germination of the SERDP-select than on the Pryor variety, whereas no different effect is exerted by the GS-HA and GN-HA.
In conclusion, the HA origin and concentration appear to affect in different ways and at different extent the germination and primary shoot and root growth of the two varieties considered.

2.2.2. Early growth data

In general, both slender wheatgrass varieties appear healthy after 21-day growth in hydroponic conditions (Figs 7-9). Almost all the seedlings have three leaves of a normal green colour.

Statistical treatment of data by one-way analysis of variance shows that, with respect to corresponding controls, the pH of the growth medium is significantly different at 0.05P and 0.001P levels in the growth media of the Pryor variety and the SERDP-select variety, respectively (Table 4). No pH variation is measured for the blanks (no plants present) during the 21-day growth period, indicating that any pH change of the growth medium is attributable to plant activity. Data in Fig.10 show that in the presence of either plant variety a significant acidification of the growth medium occurs where the HAs are present at the higher dose, with the exception of GN-HA on the SERDP-select. A high correlation exists between elongation and weight of seedlings and pH decrease for both slender wheatgrass varieties. This relation, which is generally observed when plants are grown hydroponically, may be ascribed to the release of acidic exudates and absorption of nutrients by the root system which is more evident when the medium is appropriate and possibly stimulates plant growth. At the higher HA doses, however, the pH decreases slightly in the presence of the Pryor variety and increases slightly in the presence of the SERP-select variety.

Statistical treatment by one-way analysis of variance of all experimental data (Table 4) shows that: (a) in the case of the Pryor variety, with the exception of root length, significant differences exist between the treatments and the control for any parameter measured; (b) in the case of the SERDP-select variety, the treatments are significantly different from the control only for root and shoot dry weights, whereas no statistical difference is apparent for the other parameters.

Numerical data in Table 7 (expressed in cm) and Fig. 11 (expressed in %) indicate that only the shoot length of the Pryor variety is stimulated by any HA at any concentration, whereas no significant differences exist in the other cases.

Numerical data in Table 8 (expressed in mg) and Fig. 12 (expressed in %) show that the shoot fresh weight of the Pryor variety is generally affected positively by any HA at any concentration, whereas its root fresh weight is stimulated significantly only by GN-HA at the lower concentration. No significant differences, i.e., effects of HAs, appear in the other cases,.

Numerical data in Table 9 (expressed in mg) and Fig. 13 (expressed in %) confirm those presented above for fresh weights, and show the existence of a good correlation between fresh and dry weight of seedlings of both slender wheatgrass varieties. In particular, the presence of any HA at any concentration affects positively the shoot dry weight of the Pryor variety, whereas only GN-HA at the lower concentration increases its root dry weight. However, GN-HA at both concentrations decreases the root dry weigth, and at the lower dose the shoot dry weight of the SERDP-select variety.

(2) RESEARCH PLANS FOR REMAINDER OF THE CONTRACT PERIOD

For the remainder of the contract period (20 months) research plans are the following:

(a) Experiments on the germination and early growth of the two introduced varieties Vavilov and SERP-select Siberian wheatgrass as affected by the three HAs, D-HA, GS-HA, and GN-HA.
(b) Chemical and spectroscopic characterization of HAs isolated from greenhouse soils.
(c) Experiments on the germination and early growth of the four grass varieties, alone or in combination (based on the growth differences found in the initial studies conducted at CRREL), as affected by the greenhouse soil HAs.
(d) Follow-up experiments with HA concentrations optimal to promote the growth of the four grass varieties.

(e) Correlation of the germination and seedling growth data with chemical and physico-chemical parameters of the HAs examined, in order to find out the HA parameters influencing germination and growth of the plant varieties examined.

(3) SIGNIFICANT ADMINISTRATIVE ACTIONS DURING THE PERIOD REPORTED: NONE.

(4) ANY OTHER INFORMATION : NONE.

(5) ANNEX

(A) AMOUNT OF UNUSED FUNDS REMAINING ON THE CONTRACT AT THE END OF THE PERIOD COVERED BY THE REPORT: US$ 30,000.
(B) IMPORTANT PROPERTIES ACQUIRED WITH CONTRACT DURING THIS PERIOD: NONE.
(C) METHOD OF REPRODUCTION: E-MAIL ATTACHMENTS, PHOTOCOPYING.

Table 1. Elemental composition (on moisture- and ash-free basis) of humic acids examined.

Origin of Humic acid	Moisture	Ash	C	N	H	S	O (*)
	%	%	%	%	%	%	%
Guernsey North 1	3.0	7.9	54.01 ± 0.56	4.27 ± 0.04	5.08 ± 0.12	0.50 ± 0.45	36.14
Guernsey North 2	1.7	13.9	53.82 ± 0.27	4.08 ± 0.02	5.07 ± 0.04	0.41 ± 0.05	36.62
Guernsey South 1	1.4	8.9	53.71 ± 0.17	4.11 ± 0.01	4.96 ± 0.08	0.45 ± 0.01	36.77
Guernsey South 2	2.9	4.2	55.09 ± 0.21	4.04 ± 0.02	4.66 ± 0.16	0.43 ± 0.02	35.78
Dugway 1	0.3	4.9	54.47 ± 0.06	4.81 ± 0.00	5.51 ± 0.12	0.41 ± 0.01	34.80
Dugway 2	1.6	5.1	56.36 ± 0.18	5.14 ± 0.02	6.20 ± 0.11	0.43 ± 0.00	31.87

(*) Oxygen has been obtained by difference.

Table 2. Atomic ratios (calculated on the basis of data in Table 1) of humic acids examined.

Origin of Humic acid	C/N	C/H	O/C
Guernsey North 1	14.74	0.89	0.50
Guernsey North 2	15.38	0.88	0.51
Guernsey South 1	15.23	0.90	0.51
Guernsey South 2	15.90	0.98	0.49
Dugway 1	13.20	0.82	0.48
Dugway 2	12.78	0.76	0.42

Table 3. Acidic functional group content (on moisture- and ash-free basis) of humic acids examined.

Origin of humic acid	COOH meq/g	Phen. OH meq/g	Tot. Ac. meq/g
Guernsey North	3.4	2.7	6.1
Guernsey South	3.9	1.9	5.8

Table 4. Significance level (F value) resulting from one-way Analysis of Variance (ANOVA) of all data obtained for each parameter measured distinctly for plant species.

Parameter	Slender Wheatgrass Pryor	Slender Wheatgrass SERDP-select
Germination	1.96 ^{ns}	1.42 ^{ns}
Primary root length	1.43 ^{ns}	3.78 **
Primary shoot length	13.38 ***	2.63 **
pH	2.62 *	7.69 ***
Root length	0.57 ^{ns}	1.34 ^{ns}
Shoot length	5.85 ***	1.97 ^{ns}
Root fresh weight	3.60 **	1.55 ^{ns}
Shoot fresh weight	6.58 ***	1.98 ^{ns}
Root dry weight	2.49 *	4.59 **
Shoot dry weight	5.77 ***	2.93 *

*** 0.001; ** 0.01 P; * 0.05 P; ns: nonsignificant

Table 5. Effect of HAs at different concentrations on seed germination (percentage of germinated seeds ± standard error for five replicates) measured immediately before transplanting.

Treatment	Slender Wheatgrass Pryor	Slender Wheatgrass SERDP-select
Control (H_2O)	85.5 ± 3.98	85.0 ± 3.00
D-HA		
10 mg/L	86.2 ± 3.74	93.8 ± 2.32
100 mg/L	93.6 ± 0.92	94.9 ± 2.87
GS-HA		
10 mg/L	90.2 ± 3.71	87.6 ± 1.93
100 mg/L	88.7 ± 1.02	91.5 ± 2.53
GN-HA		
10 mg/L	92.8 ± 1.87	91.6 ± 3.20
100 mg/L	79.7 ± 4.11	89.7 ± 2.08

Table 6. Effect of HAs at different concentrations on the length (cm ± standard error for five replicates) of primary root and primary shoot of germinated seeds.

Treatment	Slender Wheatgrass Pryor		Slender Wheatgrass SERDP-select	
	Root	Shoot	Root	Shoot
Control (H_2O)	2.5 ± 0.1	1.4 ± 0.1	2.5 ± 0.1	1.7 ± 0.1
D-HA				
10 mg/L	2.4 ± 0.2	1.7 ± 0.1	2.9 ± 0.1	2.0 ± 0.1
100 mg/L	2.3 ± 0.1	1.5 ± 0.04	2.7 ± 0.1	2.0 ± 0.1
GS-HA				
10 mg/L	2.5 ± 0.1	1.8 ± 0.04	2.6 ± 0.1	1.9 ± 0.1
100 mg/L	2.6 ± 0.1	1.8 ± 0.1	2.5 ± 0.1	2.0 ± 0.1
GN-HA				
10 mg/L	2.8 ± 0.1	2.0 ± 0.02	2.9 ± 0.1	2.1 ± 0.1
100 mg/L	2.7 ± 0.1	2.0 ± 0.1	3.0 ± 0.1	2.1 ± 0.1

Table 7. Effect of HAs at different concentrations on the length (cm ± standard error for five replicates) of shoots and roots measured after 21-day growth of seedlings.

Treatment	Slender Wheatgrass Pryor		Slender Wheatgrass SERDP-select	
	Roots	Shoots	Roots	Shoots
Control (H_2O)	20.4 ± 0.4	4.3 ± 0.1	18.7 ± 1.7	4.3 ± 0.3
D-HA				
10 mg/L	18.4 ± 1.3	5.7 ± 0.2	19.8 ± 0.3	4.0 ± 0.1
100 mg/L	20.7 ± 0.9	5.8 ± 0.2	22.1 ± 1.0	4.5 ± 0.3
GS-HA				
10 mg/L	20.3 ± 1.8	5.1 ± 0.2	19.9 ± 0.8	3.9 ± 0.2
100 mg/L	19.4 ± 1.2	5.6 ± 0.3	21.9 ± 0.8	4.8 ± 0.4
GN-HA				
10 mg/L	21.2 ± 0.9	5.3 ± 0.2	19.0 ± 0.8	3.6 ± 0.2
100 mg/L	20.6 ± 0.8	6.2 ± 0.3	19.1 ± 1.4	3.9 ± 0.2

Table 8. Effect of HAs at different concentrations on the fresh weight (mg ± standard error for five replicates) of shoots and roots measured after 21-day growth of seedlings.

Treatment	Slender Wheatgrass Pryor		Slender Wheatgrass SERDP-select	
	Roots	Shoots	Roots	Shoots
Control (H_2O)	29.5 ± 3.1	53.9 ± 5.2	23.9 ± 1.5	47.6 ± 5.9
D-HA				
10 mg/L	33.3 ± 1.2	82.9 ± 3.9	23.8 ± 1.9	40.5 ± 2.4
100 mg/L	35.6 ± 2.2	85.7 ± 5.5	21.6 ± 2.2	51.5 ± 5.2
GS-HA				
10 mg/L	31.1 ± 1.8	73.2 ± 5.4	24.3 ± 1.6	44.1 ± 3.8
100 mg/L	28.1 ± 3.2	99.1 ± 12.2	24.6 ± 1.2	61.1 ± 6.5
GN-HA				
10 mg/L	43.4 ± 3.2	76.2 ± 3.9	18.0 ± 3.4	34.4 ± 3.7
100 mg/L	27.7 ± 2.8	110.6 ± 4.4	18.2 ± 1.9	49.5 ± 8.1

Table 9. Effect of HAs at different concentrations on the dry weight (mg ± standard error for five replicates) of shoots and roots measured after 21-day growth of seedlings.

Treatment	Slender Wheatgrass Pryor		Slender Wheatgrass SERDP-select	
	Roots	Shoots	Roots	Shoots
Control (H_2O)	2.8 ± 0.2	10.4 ± 0.9	2.3 ± 0.2	8.6 ± 0.9
D-HA				
10 mg/L	3.0 ± 0.1	14.5 ± 2.0	1.9 ± 0.1	7.1 ± 0.3
100 mg/L	3.2 ± 0.2	15.1 ± 0.9	2.2 ± 0.1	9.4 ± 0.8
GS-HA				
10 mg/L	2.7 ± 0.1	12.5 ± 0.7	2.2 ± 0.1	8.3 ± 0.7
100 mg/L	3.0 ± 0.1	19.1 ± 1.0	2.5 ± 0.2	10.4 ± 1.1
GN-HA				
10 mg/L	3.5 ± 0.2	12.5 ± 0.7	1.5 ± 0.2	5.9 ± 0.6
100 mg/L	2.9 ± 0.1	18.5 ± 1.5	1.7 ± 0.1	8.1 ± 0.7

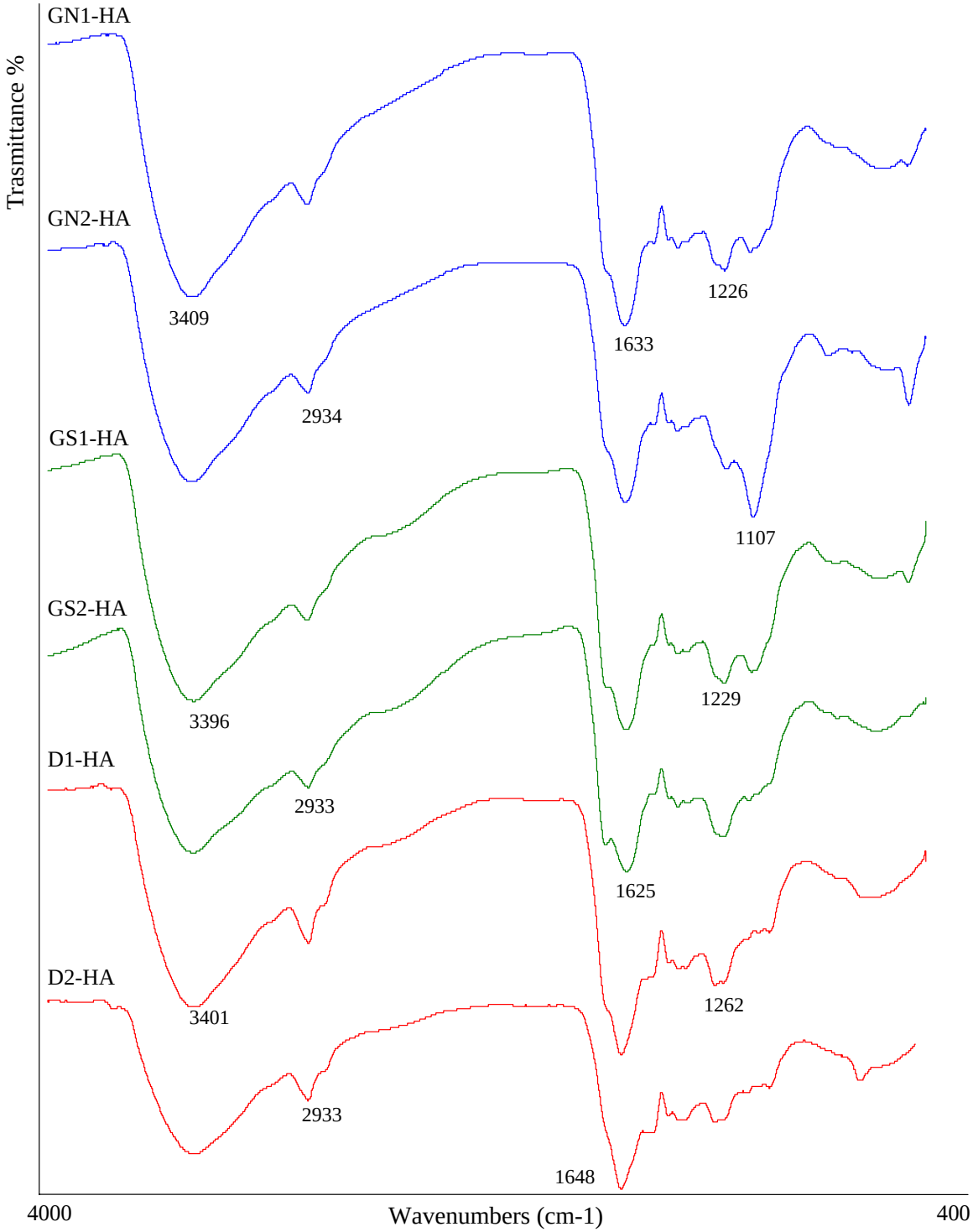


Figure 1. FT IR spectra of HAs examined.

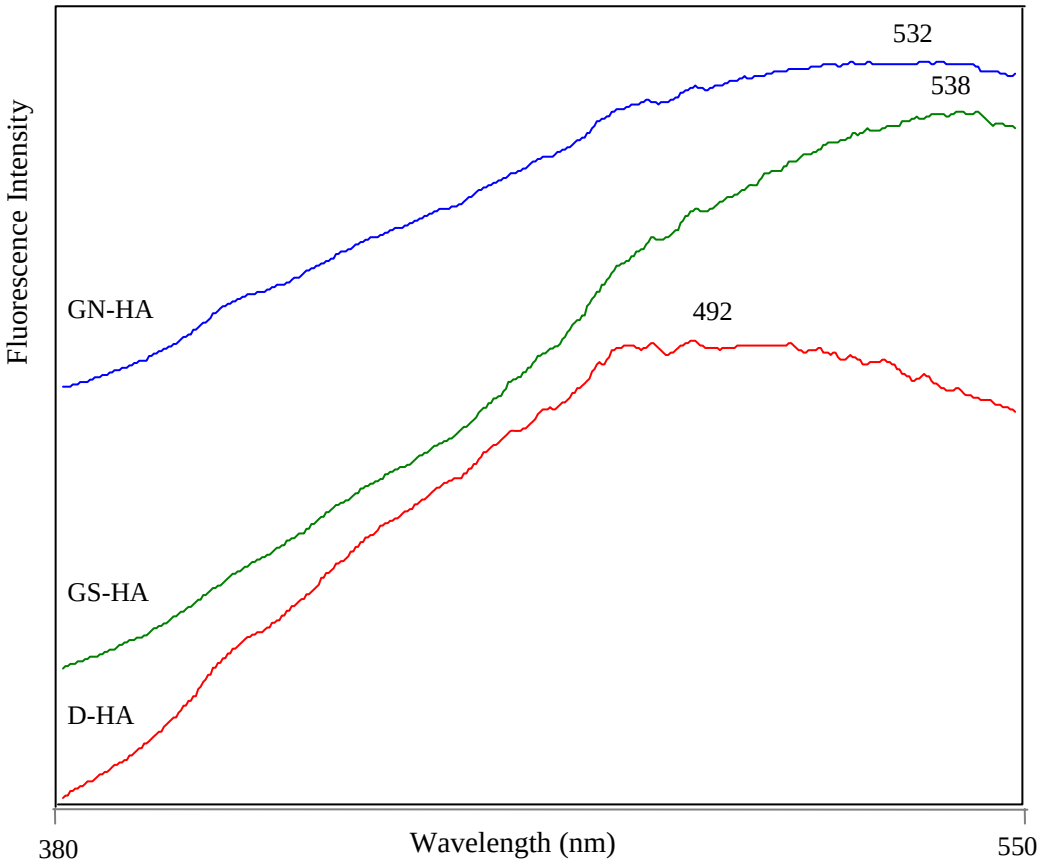


Figure 2. Fluorescence emission spectra of humic acids examined.

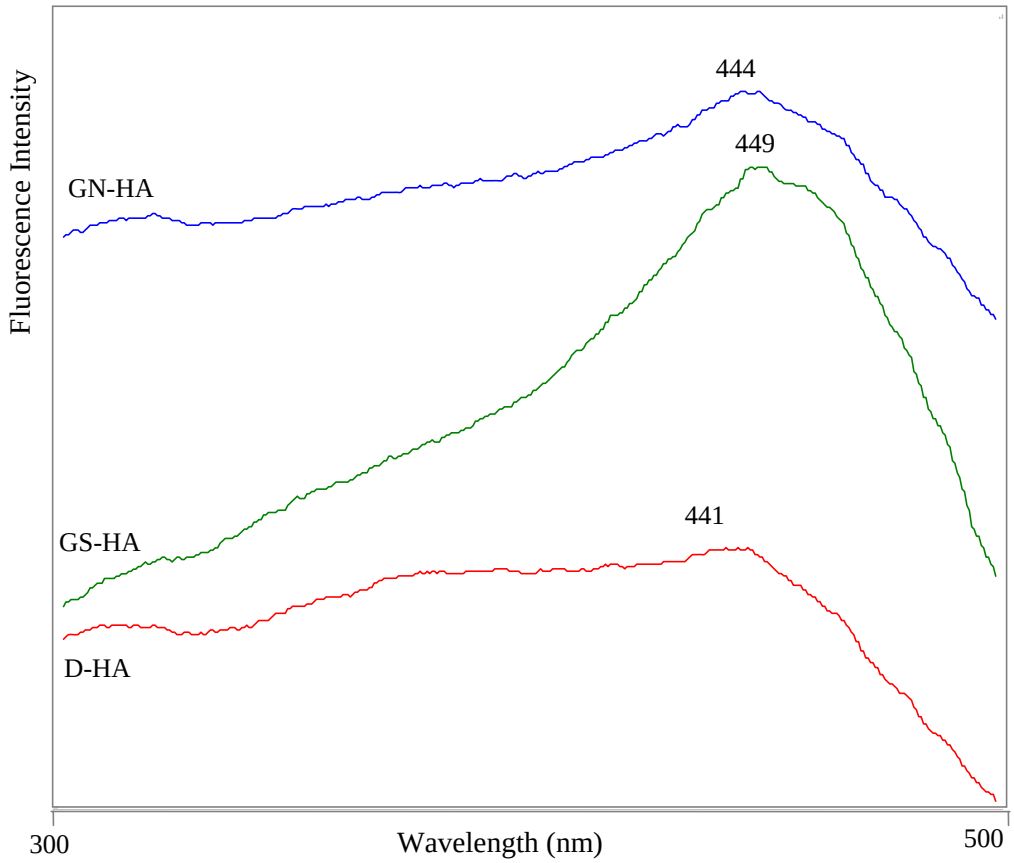


Figure 3. Fluorescence excitation spectra of humic acids examined.

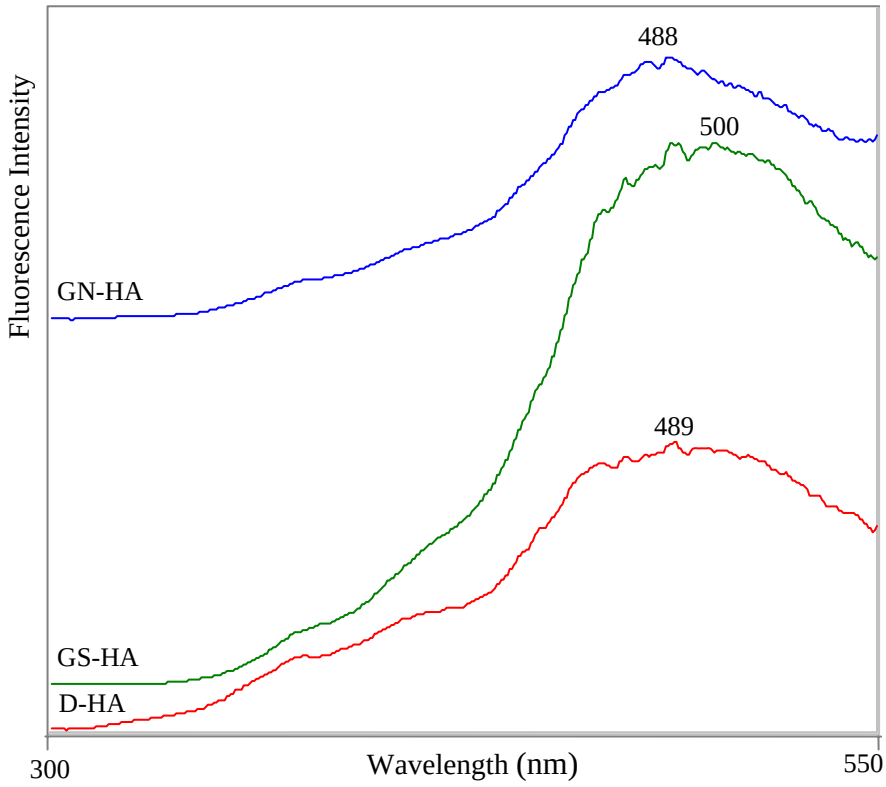


Figure 4. Fluorescence synchronous scan spectra of humic acids examined.

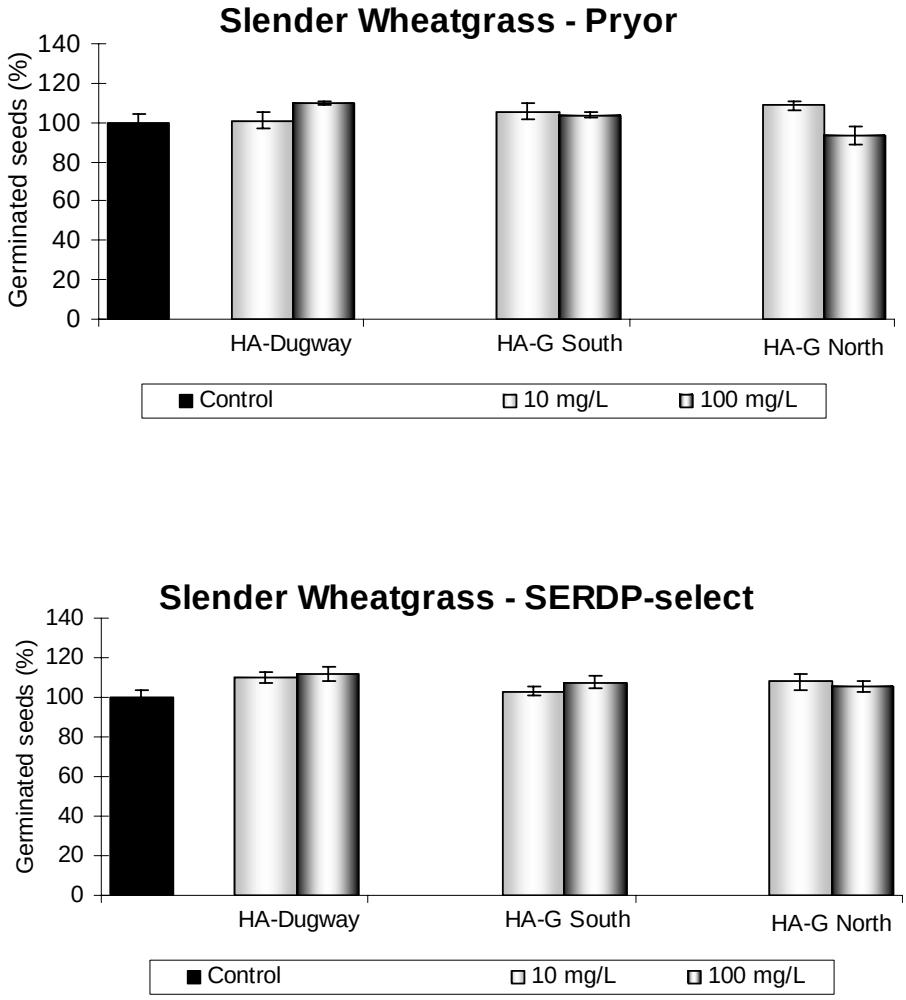
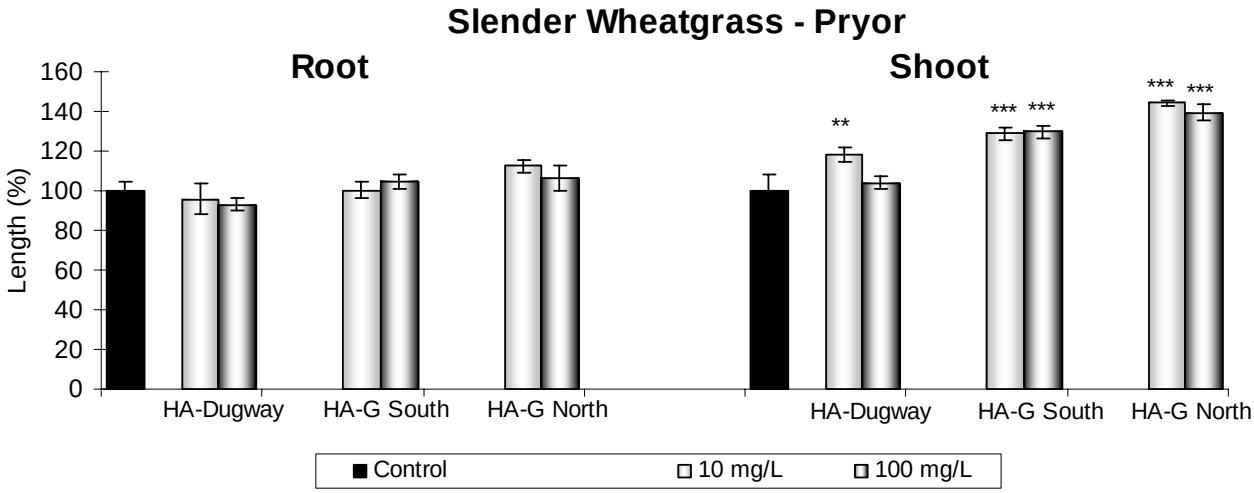
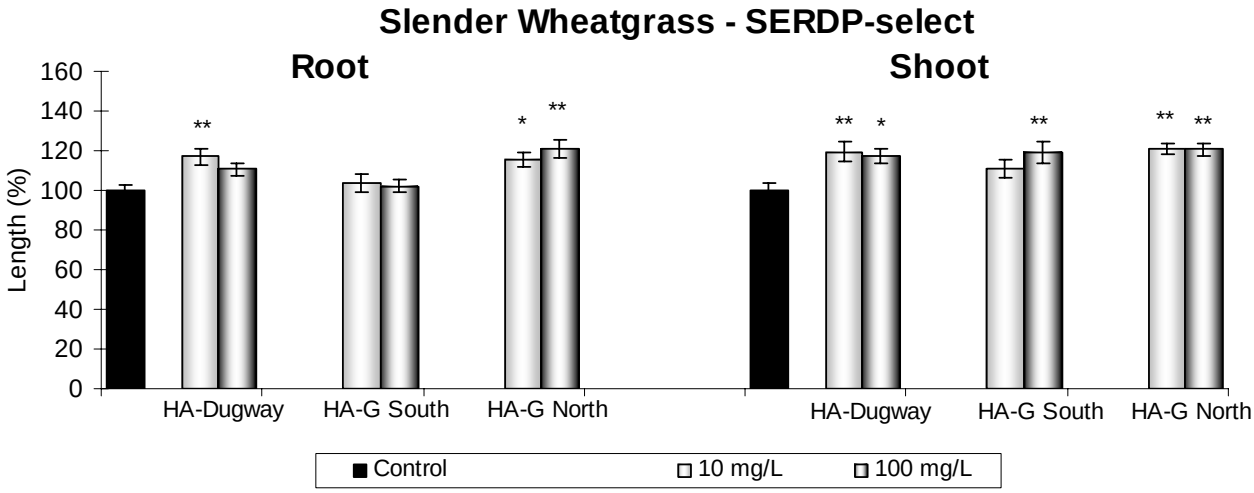


Figure 5. Effect of HAs at different concentrations on the number of germinated seeds expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 5 replicates.





*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, according to the LSD test.

Figure 6. Effect of HAs at different concentrations on the primary shoot and root length of germinated seeds, expressed as percentages of control treatment (100 %). The vertical line on each bar indicates the standard error for 5 replicates.

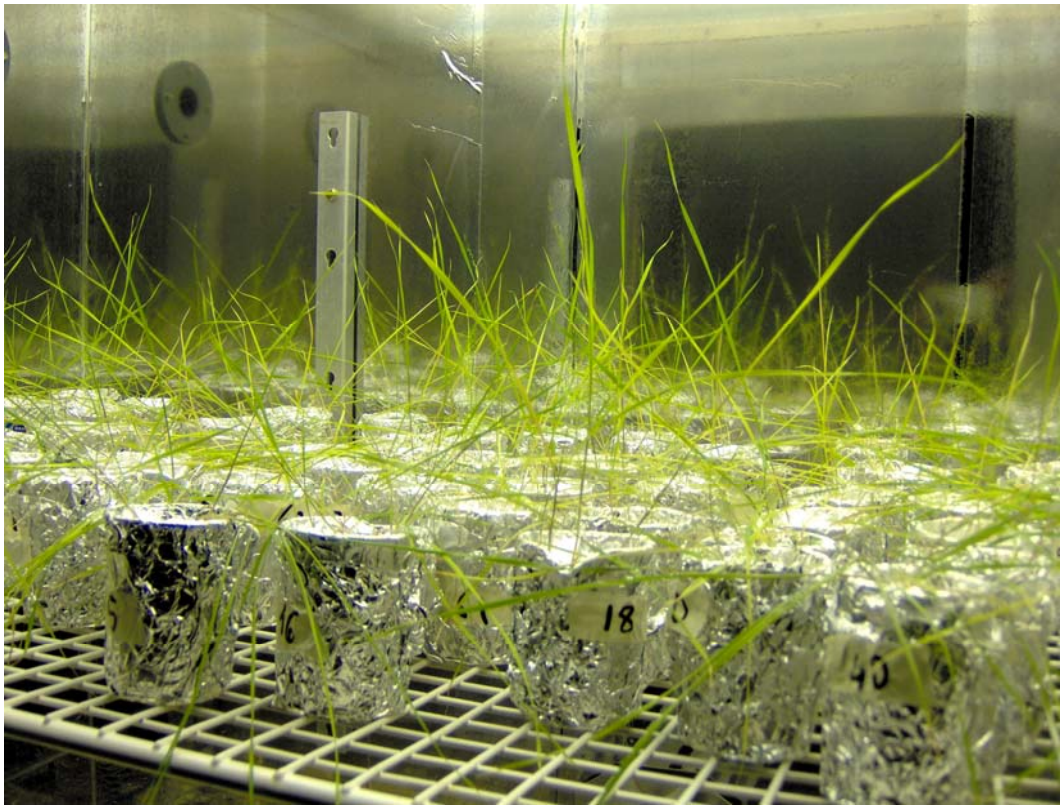
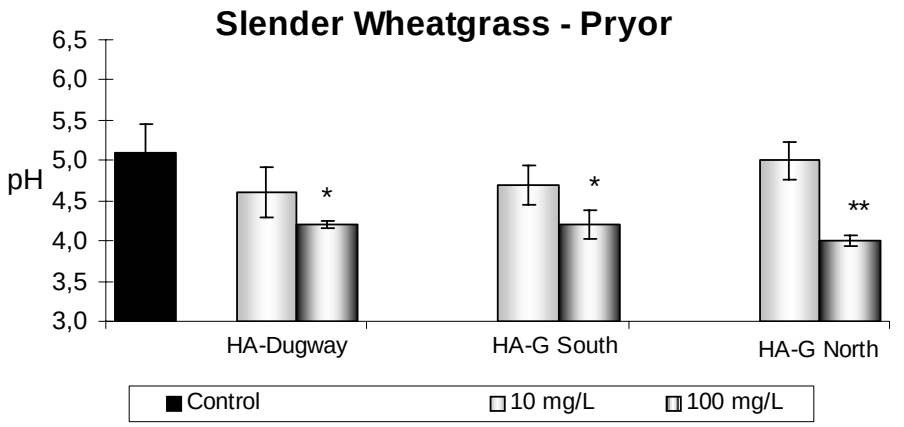
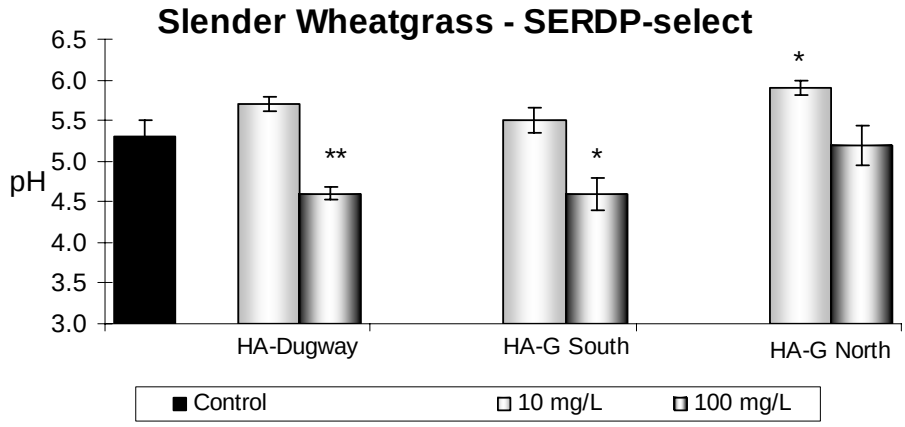


Figure 7. Seedlings of slender wheatgrass varieties after 21-day growth in the Phytotron growth chamber.

Figure 8. Seedlings of 21-day grown slender wheatgrass Pryor treated with GS-HA at 100 mg/L (top) and control (bottom).

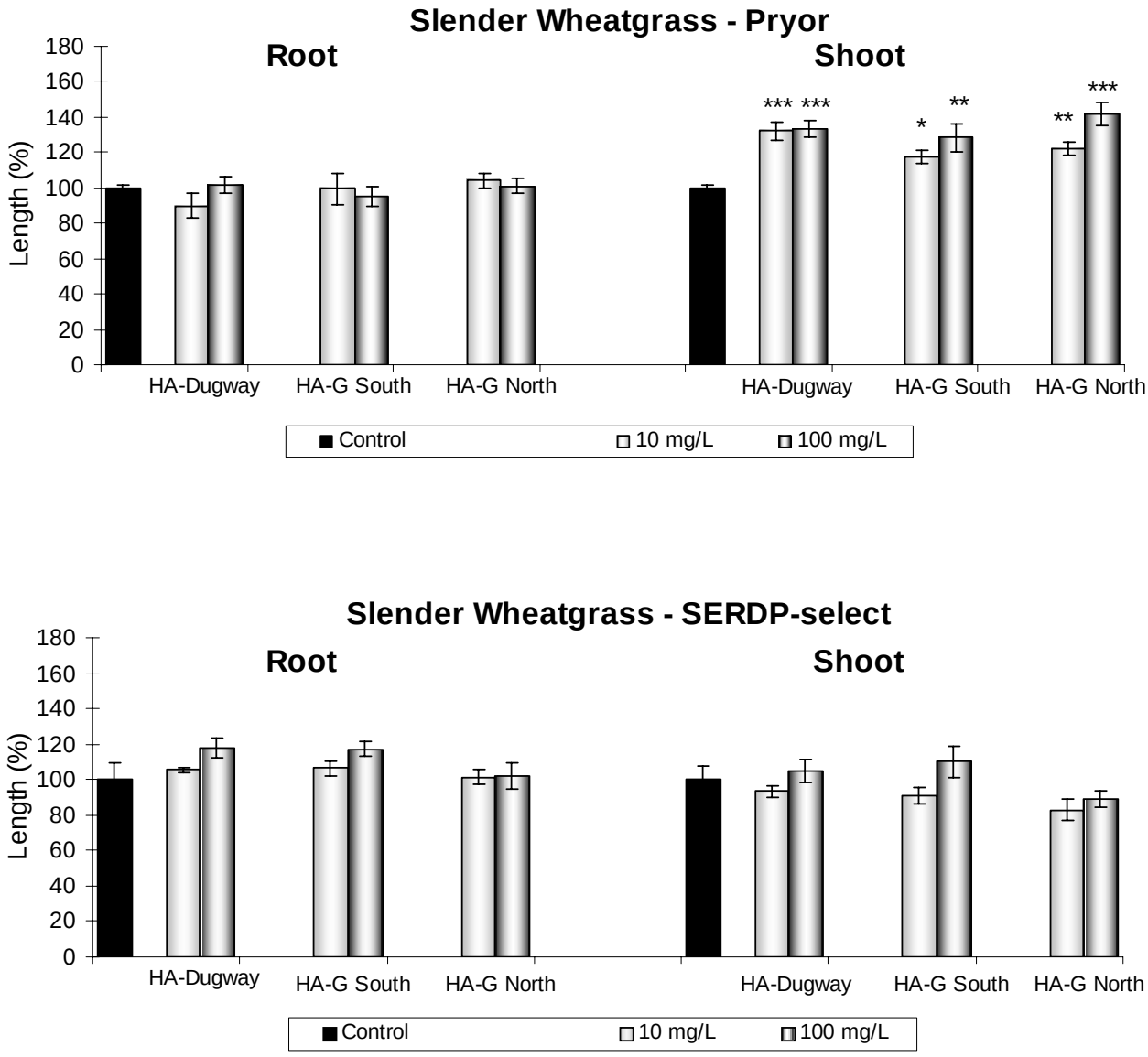
Figure 9. Seedlings of 21-day grown slender wheatgrass SERDP-select treated with GS-HA at 100 mg/L (left) and control (right).





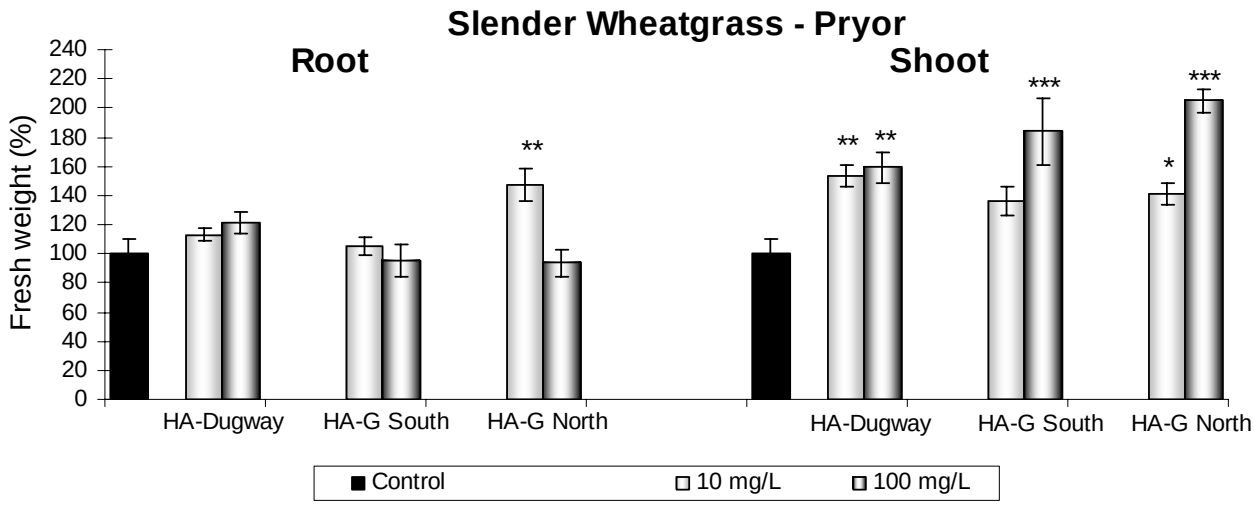
*** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05, according to the LSD test.

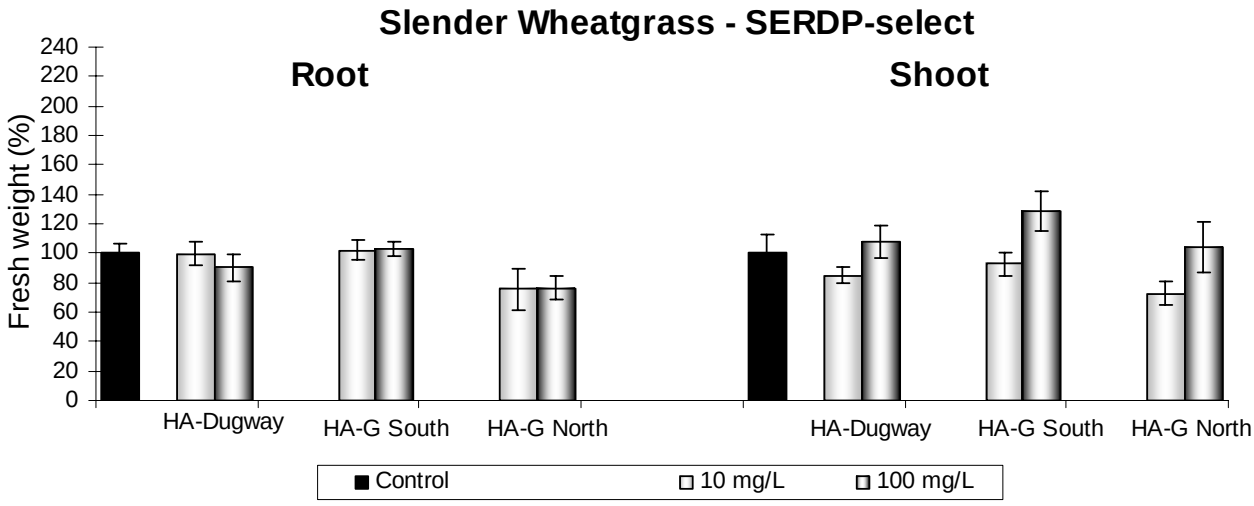
Figure 10. Effect of HAs at different concentrations on the pH value of growth medium measured after 21-day growth of seedlings. The vertical line on each bar indicates the standard error for 5 replicates.



*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, according to the LSD test.

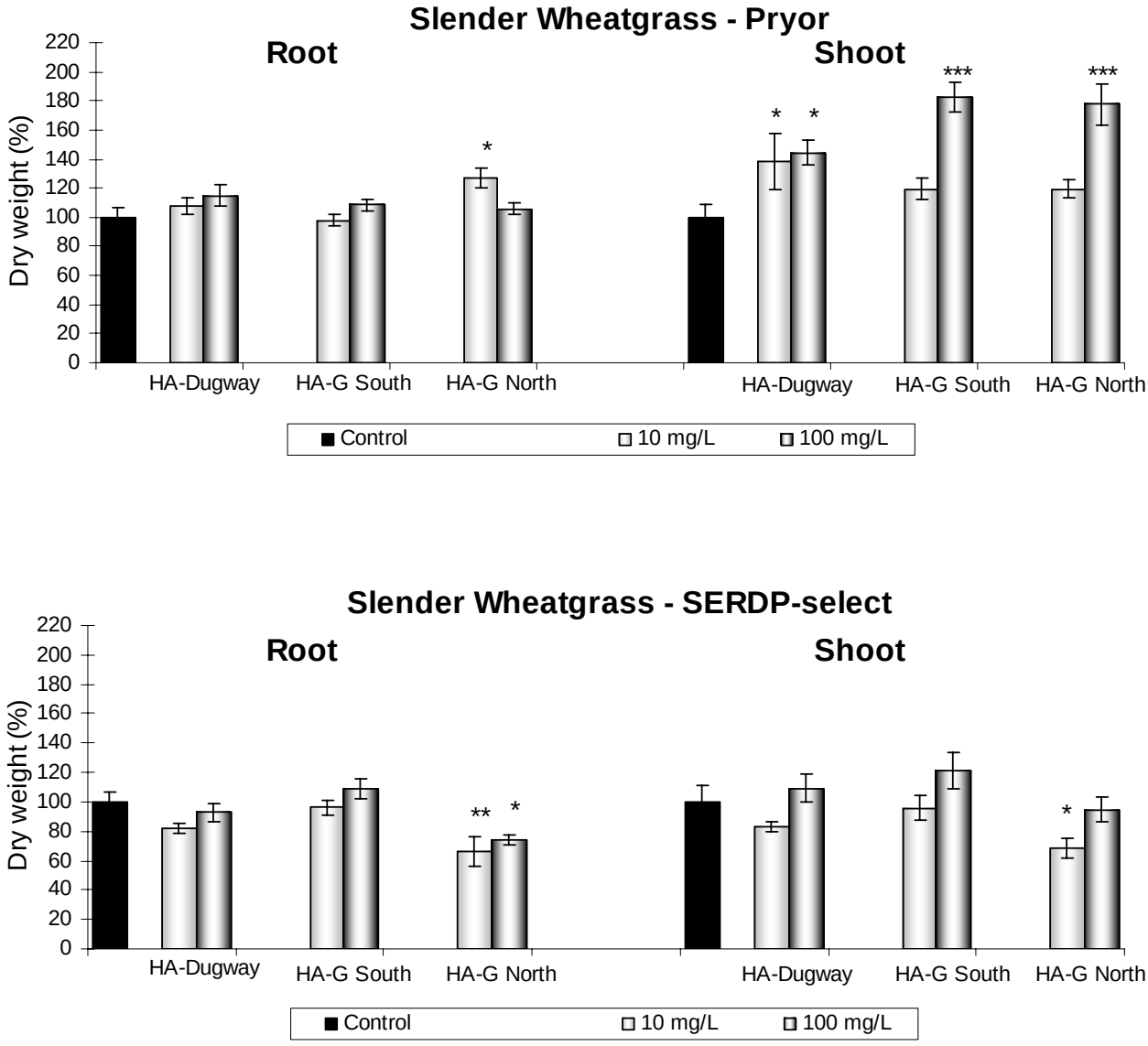
Figure 11. Effect of HAs at different concentrations on the length of shoots and roots expressed as percentages of control treatment (100 %) measured after 21-day growth of seedlings. The vertical line on each bar indicates the standard error for 5 replicates.





*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, according to the LSD test.

Figure 12. Effect of HAs at different concentrations on the fresh weight of shoots and roots expressed as percentages of control treatment (100 %) measured after 21-day growth of seedlings. The vertical line on each bar indicates the standard error for 5 replicates.



*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, according to the LSD test.

Figure 13. Effect of HAs at different concentrations on the dry weight of shoots and roots expressed as percentages of control treatment (100 %) measured after 21-day growth of seedlings. The vertical line on each bar indicates the standard error for 5 replicates.